Instruction Manual

VDPro® SIV FA Reagent

CAT.NO. RS-SIV-11

1. INTRODUCTION

Swine Influenza Virus (SIV) immunofluorescence assay (FA) reagent is designed to detect an antigen in fixed tissue by an immunofluorescence technique. The technique uses the monoclonal antibody directed against SIV in the tissue.

2. CONTENTS

Reagents		50 tests
1)	1X Anti-SIV MAb	8㎖ X 1
2)	1X FITC Anti-Mouse Conjugate	8㎖ X 1
3)	10X Washing Buffer	120㎖ X 1
4)	FA Mounting Fluid	3㎖ X 1
5)	User Manual	1 copy

3. MATERIALS

1) Glass microscope slide and cover slip
2) Cryo-microtome or microtome
3) Microscope with filters which are appropriate for FITC detection

4. WASHING BUFFER PREPARATION

Dilute with deionized water 1:10 before use to obtain 1X Washing Buffer. (e.g. 10 ㎖ 10X Washing Buffer + 90 ㎖ water)

Note: All diluted 1X Washing Buffer must be used within 3 days and store at 4℃ until used.

5. SAMPLE TREATMENT

❖ **Sample collection**

Samples were prepared from fresh lung, trachea and so on. Also, these tissues should be fresh samples harvested quickly after necropsy.

❖ **Frozen section**

Cover with OCT compound collected tissue in mold and freeze at -20℃.

Cut the frozen tissue to 4~6um thickness at -10~-15℃. 4~6um thickness is suited to FA test. However, regulate depending on lab situation.

❖ **Drying and Fixation**

Dry slides for 5 minutes at room temperature.

Incubate them for 5~10 minutes at room temperature with cold acetone and wash the slide three times with wash solution.

6. FA TEST (PROCEDURE)

1) Prepare tissue slides.
2) Add 2~4 drops (50~100㎕) of 1X Anti-SIV MAb to cover tissue section
3) Incubate the slides at room temperature for 30~60 minutes in humid chamber.
4) Discard the solution and blot from slides by using absorbent filter paper completely. Do not allow it to dry.
5) Wash slide 3 times for 3 minutes each with 1X Washing Buffer.
6) Add 2~4 drops (50~100㎕) of 1X FITC Anti-Mouse Conjugate to cover tissue section. Incubate the slides at room temperature for 30~60 minutes in humid chamber.
7) Wash slide 3 times for 3 minutes each with 1X Washing Buffer. Blot excess washing buffer from slides with absorbent filter paper completely.
8) Add 2~4 drops (50~100㎕) of FA Mounting Fluid to the slides. Cover it with coverslip and examine the tissue sections under a dark field microscope.

Notes

- ❖ Modify washing number and time according to lab condition or sample type because fluorescence density of sample could be different.
- ❖ If nonspecific fluorescence detect on background can use 2-fold diluted primary antibody or secondary antibody.
- ❖ Slide background was detected with red because 1X FITC Anti-Mouse Conjugate contains control stain reagent.

7. INTERPRETATION

1) Infected cells are detected with bright green on dark green or dark gray background.
2) Bright green fluorescence will detect in cytoplasm or nucleus of infected cells.
3) Background color or density varies according to the form, quality of species. It is detected with gray, green, yellow, red, brown, but is differentiated from positive reaction (bright green).
4) For accuracy of test, it should be performed internal positive tissue slides control.

Confirmation and Additional test

- ❖ If clinical symptom and FA test is positive, it concludes SIV positive. However, histopathological test, virus isolation and serological test must be performed.
- ❖ If clinical symptom is suspect and FA test is negative, it should virus isolation or virus gene detection.
- ❖ In case of vaccinated farm, consider that typical clinical symptom or lesions on necropsy were not mostly presented.

Median Diagnostics Inc.
878, Sunhwan-daero, Dongnae-myeon, Chuncheon-si, Gangwon-do, 24399, Republic of Korea
Tel: +82 (0)33 244 0100 / Fax: +82 (0)33 244 4634
E-mail: median@mediandx.com